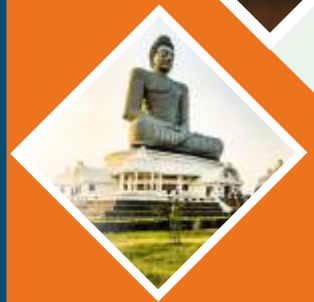
World Embryologist Day & 6th TSAP- Twin state Embryologist meet

SOUVENIR

22 JULY 2023

Hotel Green Park,
Hyderabad



Editorial Board

www.embryoconnect.net

Uniting the Global Embryology Community, Inspiring Innovation

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Our mission

To facilitate connections, knowledge sharing, career advancement, and collaborative research within this dynamic community.

Connect and Collaborate:

In a rapidly evolving field like embryology, innovative tools for interaction and collaboration are essential. That's where embryoconnect.net comes in. Our platform enables valuable exchanges between professionals, keeping you at the forefront of the latest breakthroughs in reproductive medicine.

Community Contributions:

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Our Marketplace exists to support the needs of our community, making it simpler for embryologists to acquire necessary resources and to recirculate items that can be beneficial to others in the field.

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embryoconnect.net is your portal to the latest news and research in reproductive medicine. Stay tuned for updates on recent advances, upcoming conferences, webinars, and other relevant events.

embryoconnect.net is more than a website. It's a dynamic community designed to support and engage everyone in the field of embryology. Whether you're an embryologist, researcher, or employer, you're an essential part of our vibrant community.

Join embryoconnect.net today and be part of our mission to **connect, collaborate, and innovate.** Because together, we can achieve so much more.

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Dear Amazing Colleagues,

When I and Durai first envisioned this project, we wanted it to be a true celebration of our collective pursuit of excellence in the world of ART. And now, it's a reality—a heartfelt tribute to the groundbreaking work that happens in our laboratories and a constant reminder of the incredible role we play in bringing new life into this world!

Can we take a moment to appreciate the remarkable progress we've witnessed in ART over the last few decades? From the incredible advancements in techniques like IVF, ICSI, and PGT to the game-changing improvements in cryopreservation and time-lapse imaging—our field has been on a fantastic journey of growth!

As clinical embryologists, our mission goes beyond achieving pregnancies; we strive to optimize outcomes and ensure the well-being of both parents and embryos. And our hard work has paid off! With improved culture media, advanced embryo selection algorithms, and the practice of single embryo transfer, we have minimized complications and boosted pregnancy health!

But, hey, with great advancements come great responsibilities, right? We've got some ethical and regulatory challenges on our plate. But fear not! We are committed to striking the perfect balance between reproductive success and patient autonomy. Ensuring informed consent, responsible technology use, and patient well-being is at the heart of everything we do.

Looking into the future, the prospects are simply awe-inspiring! With gene editing technologies like CRISPR-Cas9 on the horizon, we might be able to tackle genetic defects and prevent inheritable diseases! And let's not forget about the amazing potential of artificial intelligence, machine learning, and big data analytics. Embracing these cutting-edge technologies will undoubtedly take our success rates to even greater heights!

But it's not just about the tech; it's about teamwork too! By collaborating with experts from different fields, we can make groundbreaking discoveries and revolutionize reproductive medicine!

I want to take a moment to express my heartfelt appreciation for all the incredible advancements and unwavering dedication of my fellow embryologists. You are all rock stars, and it's an absolute honor to work alongside you!

So, here's to the bright future of clinical embryology! Let's continue to innovate, collaborate, and uphold the highest ethical standards in our journey to bring hope and joy to countless families seeking to build their dreams.

Cheers to all of you! With boundless excitement and gratitude,

Dr. Chekuri Suvarchala Vardhan

Editor-in-chief
Co-Founder of this Souvenir
MSc in Clinical Embryology (Monash, Australia),
MSc in Clinical Embryology (Leeds, UK), MSc in Genetics
Scientific Director ZIVA Embryology and Fertility Institute, Hyderabad

Dear colleagues and ART enthusiasts,

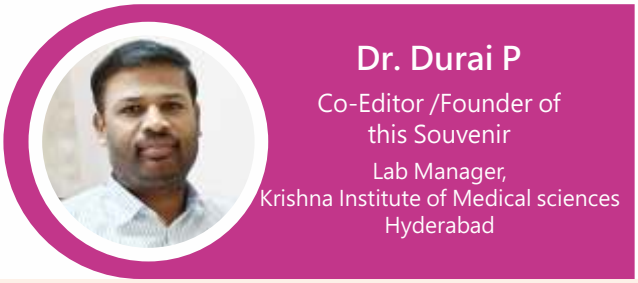
It brings me immense joy and pride to introduce this remarkable souvenir, which embodies the collective wisdom and expertise of leading professionals from Telangana and Andhra Pradesh. As the founder of this cherished collection, my vision was to create a platform that fosters collaboration, promotes excellence, and empowers continuous improvement in our practices.

Each day we step into our labs, we aren't merely working with cells and culture media; we're trusted with people's dreams. So let's embrace QC as our ally, not an adversary, and let it guide us towards providing the best care possible to our patients.

I extend my heartfelt gratitude to all the contributors, supporters, and co-creators of this endeavour. Your dedication has made this dream a reality, and I am honoured to be part of this remarkable community.

Let's explore, learn, innovate, and continue to change lives.

With profound gratitude and excitement,



Dr. Durai P
Co-Editor /Founder of this Souvenir
Lab Manager,
Krishna Institute of Medical sciences
Hyderabad

As the Co-Editor of this special memento, I'm thrilled to present it to our committed community of embryologists. This compilation encapsulates insights from eminent experts in Telangana and Andhrapradesh, showcasing our collective drive for perfection in Assisted Reproductive Technology. Each day we step into the lab, we're not just implementing processes, but weaving dreams with Quality Control as our ally. This souvenir is a salute to that alliance and our relentless quest for enhancement. I hope you find it insightful and inspiring!

Regards
Dr Prasad M
Co-Editor
Lab Director, Anu Test Tube Baby Centre

Dear Esteemed Colleagues,

I am absolutely delighted to present to you this extraordinary collection that embodies our collective pursuit of excellence in the world of ART. Our journey in unravelling the mysteries of life continues each day, and in this endeavour, let us celebrate the invaluable role of Quality Control as our trusted guide. I trust this memento will illuminate your path, fuel your curiosity, and strengthen our shared resolve.

This memento is more than just a token; it's a beacon of light that will illuminate your path, fuel your curiosity, and reinforce our unwavering resolve as members of the TSAP Embryologist Forum. Together, we have the power to weave wonders, and I couldn't be prouder to have you all by my side.

With heartfelt cheer and enthusiasm,

"Speculation and the exploration of ideas beyond what we know with certainly are what lead to progress"

Em Swaminathan D
MD/CEO, Jones Academy of Clinical Embryology, Chennai
Former President of the ACE India
Scientific Director,
Santhathi centre for reproductive medicine

"Coming together is a beginning, keeping together is progress, working together is success" If we continue to think the way we have always thought, we will continue to get what we have always got. Is it enough?? A lovely quote on why we must continue to share our experiences as embryologists, on this wonderful Telangana & Andhra Pradesh ACE platform. I take this opportunity to extend my heartfelt best wishes & congratulations to all the members of Telangana & Andhra Pradesh ACE for bringing together like-minded, dedicated embryologists of Telangana & Andhra Pradesh and continually updating & enriching them with expert talks, panel discussions & hybrid conferences on key topics in Reproductive medicine. organizers & the event all success. As the world celebrates embryologists day once again, Telangana & Andhra Pradesh ACE comes forward with a hybrid meeting of great significance on quality control in the IVF lab. We are witnessing increasing complexity of day-to-day procedures in our lab & this requires dedicated quality control & assurance implementation to safeguard us, our patients embryos & our success. This hybrid meet will update & strengthen your knowledge base in this area. I wish the organizers & the event all success.

Dr. Sapna Srinivas,
LAB Director,
Mamata Fertility Hospital,
Secunderabad

As an expert in the field, it brings me immense joy to share this collection of insights and best practices in ART with you all. This keepsake is a testament to our shared passion for excellence and our dedication to maintaining the highest standards of quality control in our work. May it inspire, challenge, and guide us as we continue to transform lives and create miracles in our laboratories every day.

Best Regards
Dr. Charulata Chatterjee,PhD
Scientific head and
consultant embryologist,
Ferty9 Fertility Center.

Dear Colleagues,

Delighted to present this keepsake filled with expert insights, a testament to our shared dedication in the field of ART. As we navigate this intricate path daily, let's view Quality Control as our guiding star, steering us as we nurture dreams into reality. May this souvenir inspire you further in our noble journey. Together, let's continue creating miracles

Warm Regards,
Dr Akash A
ESHRE Certified
Scientific Director
Hegde Fertility Centre Hyd.

Dear Colleagues,

It's a privilege to share with you this collection, embodying our mutual pursuit of excellence in ART. As we unravel the mysteries of life daily, let's acknowledge Quality Control as our trusted guide, assisting us in transforming dreams into reality. I trust this memento will illuminate your path, fuel your curiosity, and strengthen our shared resolve.

Kind Regards
Mohammed Nissar
Co-founder,
Angel fertility centre
Hyderabad

Role of Electronic Witnessing System in an ART Laboratory

Rachel Chin
Clinical Application Manager (APAC)
Coopersurgical, Malaysia

When a patient embarks on a life changing journey with ART treatment, they expect to be assured that everything is being taken care of. Apart from a positive treatment outcome, fertility patients want high quality in their fertility care from the clinic.

To achieve and maintain the highest level of patient care and to drive clinical efficacy, it becomes crucial for fertility clinics especially the ART laboratory to adopt a strong quality management system (QMS) for quality assurance, continual improvement, and consequent increase in customer satisfaction.

IVF procedures in recent years are getting more complex with a higher number of manipulation steps. As a result, each embryologist is handling more manipulation steps in which the risk of error becomes high with the potential loss of a complete cycle for the patient. The impact of various activities and the possible risks makes it necessary to ensure the safety and reproducibility of all methods. Having an electronic witnessing system & a lab management tool can help ART clinics raise the standard of care & maintain high quality with respect to lab management.

Over the years, the evidence is clear that manual witnessing is incapable to overcome all identity errors as manual monitoring and recording keeping lacks accuracy and is highly labor intensive. Since 2007, at the forefront of the industry movement towards increased visibility and accountability, CooperSurgical designed RI Witness TM , an electronic witnessing and complete ART lab management system that gives the infertility specialists an increased confidence in their everyday practises. It is now the world's most established and trusted ART electronic witnessing system, installed in over 40 countries across six continents.

Electronic witnessing system comes in the form of radio frequency identification (RFID) or barcoding system. Unlike barcoding system, RFID witnessing system such as RI Witness requires very minimal active participation from people. This makes it to be least vulnerable to human errors or intervention which is an ideal fit for an IVF laboratory as it is highly robust and an efficient error prevention system. An electronic witnessing system (EWS) also significantly
streamlines the witnessing process by reducing the time required for witnessing while at the same time allows an increase in the number of types of witnessing sessions per patient.

IVF laboratory that has EWS such as RI Witness TM has a strong advantage in traceability as the system only allows the next step in the procedure if all criteria are met. This is a great advantage as it ensures the adherence of SOPs by all lab personnel. When RI Witness operates, it automatically records all laboratory data. The built-in analytical function of the system tracks and analyzes data, as well as creates reports such as total procedure time per location, total procedure time between operators, etc. Such detailed insight allows the lab manager to identify areas for improvement within the laboratory and also all helps in planning for lab personnel upskilling and training. The advanced analytic feature of the RI Witness system - Witness IQ allows data from multiple clinics to be seen in one report. It also allows the lab manager to see procedure durations over larger periods and how durations change within busy periods, staffing levels, or procedure changes in the lab.

Implementing 6S Methodology in Assisted Reproductive Technology (ART) Laboratories

Dr Prasad M
Anu test tube Centre,
Hyderbad
Lab Director, Anu Test Tube Baby Centre

Delighted to present this keepsake filled with expert insights, a testament to our shared dedication in the field of ART. As we navigate this intricate path daily, let's view Quality Control as our guiding star, steering us as we nurture dreams into reality. May this souvenir inspire you further in our noble journey. Together, let's continue creating miracles

Understanding the 6S Methodology

What is 6S ?

The 6S methodology is a systematic approach to workplace organization and standardization. It comprises six components:

1.Sort (Seiri): The process starts by sorting out necessary items from unnecessary ones. In an ART laboratory, this might involve removing excess equipment or supplies that are not regularly used and may be taking up valuable space.
2.Set in Order (Seiton): This step involves arranging necessary items so they are readily accessible and easy to find. It enhances workflow efficiency by reducing the time spent searching for required items.
3.Shine (Seiso): Regular cleaning and maintenance of the workspace are essential. In ART laboratories, this includes the maintenance of laboratory equipment and keeping the area clean to minimize the risk of contamination.

4.Standardize (Seiketsu): Standardizing workflows and procedures ensures that the first three S's are performed consistently and predictably. Standard Operating Procedures (SOPs) play a critical role in this stage, providing clear instructions for various tasks in the ART lab.
5.Sustain (Shitsuke): Sustaining the improvements made requires discipline and commitment. Regular audits, reviews, and training can help ensure the practices of 6S are deeply ingrained in the laboratory's culture.
6.Safety (The 6th 'S'): The additional S, Safety, is about creating a safe work environment. In ART laboratories, this could involve managing biological hazards, proper handling, and storage of chemicals, and ensuring correct usage of laboratory equipment.
Benefits of 6S in ART Laboratories Improved Efficiency: By sorting and setting items in an order, the workflow becomes more efficient. Laboratory staff can easily find what they need, reducing wasted time and minimizing disruption to critical procedures.
Enhanced Safety: A clean, well-organized lab is a safer lab. Implementing safety measures can reduce the likelihood of accidents and mishandling of delicate samples.
Reduced Errors: Standardization reduces the chances of errors, as every procedure is clearly defined and consistently executed. It also allows easier identification and rectification of mistakes when they do occur.

The Kaizen Carnival: Celebrating Continuous Improvement in ART Labs

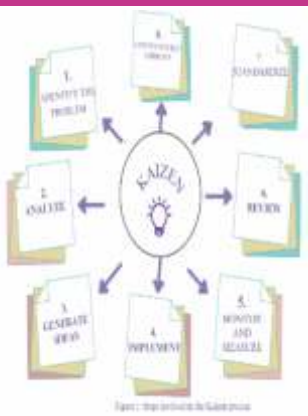
Dr Durai Ph D

Lab Manager, Chief Embryologist
Department of Reproductive Medicine
Krishna Institute of Medical Sciences
Hyderabad

Welcome to the world of ART labs, where miracles happen every day! Here, the joy of life literally starts from scratch (or, to be more precise, a single cell). But have you ever wondered how these laboratories can continuously improve their processes, minimize errors, and increase success rates, all while maintaining an atmosphere of precision and control that makes a Swiss watchmaker look like a laid-back surfer dude? Well, they have a secret weapon in their arsenal: Kaizen. And no, it's not a new-age fertility technique or a secret serum. It's a management philosophy that hails from the land of sushi and samurai. So, let's delve into the quirky yet powerful world of Kaizen in ART labs!

What is Kaizen, Anyway?

Kaizen is Japanese for 'change for the better.' It's a bit like sushi - small, seemingly simple, but when consumed regularly, leads to better health (or, in our case, better work processes). It's not about transforming everything overnight like a contestant on a reality TV makeover show. Rather, it's about making tiny changes every day, which over time add up to a complete transformation



Kaizen in ART Labs: A Match Made in Test Tube

Assisted Reproductive Technology (ART) Labs are like finely-tuned orchestras, where everyone and everything must work in perfect harmony. But just as an orchestra needs regular rehearsals and fine-tuning of instruments, ART labs need continual improvement

Here, even the tiniest mishap can mess up the symphony of life. Imagine accidentally adding one extra ingredient while baking, and instead of a cake, you get a...dinosaur? (Okay, not that extreme, but you get the point!) That's where Kaizen comes in, ensuring that every part of the process is as sleek as a seal and as efficient as a Japanese bullet train.

The Kaizen Way So, how does Kaizen work its magic in ART labs? Let's take a fun little journey.

1. Employee Empowerment: Imagine a kingdom where every citizen, from the royal vizier to the humble baker, has a say in running the state. That's Kaizen for you. It turns the ART lab into a democratic space where every individual, regardless of their role, can suggest improvements. Even the quiet lab technician in the corner might come up with a genius idea to streamline processes!

2. Standardizing Procedures: Imagine doing the Cha-Cha without any steps. Chaos, right? Standardization is the choreography of the laboratory dance. It ensures everyone knows their steps, maintaining consistency and reducing chances of tripping over (aka making errors).

3.Continuous Improvement: Kaizen is a bit like a nosy neighbor, always checking in, always observing, and always looking for something new. But unlike the neighbor, it's all for a good cause – to continually spot areas that need improvement and plan changes.

4.Eliminating Waste: Got extra steps in a protocol? Too much unused resources? Redundant paperwork? Kaizen, like an expert detective, sniffs out the waste and eliminates it, ensuring the ART lab runs as smooth as butter on a hot pancake.

5.Training: And finally, Kaizen believes in learning and growing together, like a big happy family. Regular training sessions ensure every staff member is updated on the latest dance steps in the Kaizen groove.

The Happy Ending

Thanks to Kaizen, ART labs can metamorphose into streamlined, productive, miracle-creating hubs (strictly speaking in terms of scientific wonders, naturally)! It's not merely about enhancing success percentages or curbing blunders; it's about establishing...

A guide to incorporate key performance indicators in an IVF program

Dr Krishna Mantravadi
Scientific Head, Oasis Fertility

Gayathri Gedela
Sr. Embryologist,
Oasis Fertility Hyderabad

Quality management systems (QMS) and its processes requires an organisation to "maintain documented information to the extent necessary to support the operation of processes and retain documented information to the extent necessary to have confidence that the processes are being carried out as planned."
Having a well-defined process mapping, updated Standard Operating Procedures (SOP), Data collection and document control as per the SOPs, regular audit of data, benchmarking of the program as per pre-defined KPIs and if warranted trouble shooting or risk mitigation these are the essentials of a well-run TQMS program.
This article will deal with KPIs in detail.

Key Performance Indicators (KPIs)

KPIs aid in setting standards for competency and benchmarking into ART practice. KPIs help to maximise the chance of success and minimise risk.

Key Performance indicators (KPIs) are objective measures for evaluating critical healthcare domains (patient safety, effectiveness, equity, patient-centeredness, timeliness and efficiency). In the setting of a clinical laboratory, quality indicators are necessary for systematically monitoring and evaluating the laboratory's contribution to patient care (ISO15189-2012) and they represent an important element within the Quality Management System (QMS). Any KPI should be reliable

Table II PIs for identifying performance of the ART laboratory.

PI	Calculation	Benchmark value
Proportion of oocytes recovered (stimulated cycles)	no. oocytes retrieved / no. follicles on day of trigger x 100	80-95% of follicles measured
Proportion of MII oocytes at ICSI	no. MII oocytes at ICSI / no. COCs retrieved x 100	75-90%

The table above describes the KPIs related to controlled ovarian stimulation and skill of the clinician in an ART program. Of the measured follicles during folliculometry, atleast 80-95% follicles should yield oocytes and of these 75-90% should be mature metaphase2 oocytes. Oocyte retrieval of more than 95% of measured follicles and maturity in oocyte upto 90% indicates that the clinician is competent.

Table III PIs for the ART laboratory.

PI	Calculation	Competency value (%)	Benchmark value (%)
Sperm motility post preparation (for IVF and IUI)	progressively motile sperm / all sperm counted x 100	90	≥95
IVF polyspermy rate	no. fertilized oocytes with > 2PN / no. COCs inseminated x 100		<6
1 PN rate (IVF)	no. 1PN oocytes / no. COCs inseminated x 100		<5
1 PN rate (ICSI)	no. 1PN oocytes / no. MII oocytes injected x 100		<3
Good blastocyst development rate	no. good quality blastocysts on Day 5 / no. 2PN/2PB oocytes on Day 1 x 100	≥30	≥40

PN, pronucleus; PI, performance indicator; PB, polar body.

The table above describes the KPIs related to IVF program in an embryology lab. Competency is the minimum performance expected and Benchmark values are the state of art best performance levels to achieve.
Following is an example of how to interpret the values described in Vienna consensus in our ART programs. It is expected that our embryology laboratory post IVF/ICSI should atleast offer 30% good blastocysts from fertilized oocytes and a conversion rate of 40% and above indicates that the lab and the scientific team are offering the best outcomes. Blastocyst conversion rates of less than 30% is considered less and calls for trouble shooting or risk mitigation.

KPI	Calculation	Competency value (%)	Benchmark value (%)
ICSI damage rate	no. damaged or degenerated / all oocytes injected x 100	≤10	≤5
ICSI normal fertilization rate	no. oocytes with 2PN and 2PB / no. MII oocytes injected x 100	≥65	≥80
IVF normal fertilization rate	no. oocytes with 2PN and 2PB / no. COCs inseminated x 100	≥60	≥75
Failed fertilization rate (IVF)	no. cycles with no evidence of fertilization / no. of stimulated IVF cycles x 100	<5	
Cleavage rate	no. cleaved embryos Day 2 / no. 2PN/2PB oocytes on Day 1 x 100	≥90	≥99
Day 2 Embryo development rate	no. 4 cell embryos on Day 2 / no. normally fertilized oocytes* x 100	≥50	≥80
Day 3 Embryo development rate	no. eight cell embryos on Day 3 / no. normally fertilized oocytes* x 100	≥45	≥70
Blastocyst development rate	no. Blastocysts Day 5 / no. normally fertilized oocytes* x 100	≥40	≥60
Successful biopsy rate	no. biopsies with DNA detected / no. biopsies performed x 100	≥90	≥95
Blastocyst cryosurvival rate	no. Blastocysts appearing intact / no. Blastocysts warmed x 100	≥90	≥99
Implantation rate (cleavage stage)*	no. sacs seen on ultrasound / no. embryos transferred x 100	≥25	≥35
Implantation rate (blastocyst stage)*	no. sacs seen on ultrasound / no. blastocysts transferred x 100	≥35	≥60

Based on the information presented here, each laboratory should develop its own set of KPIs founded on laboratory organization and processes, and develop a systematic, transparent and consistent approach to data collection and analysis and calculation of KPIs.

Key Performance indicators of an ART lab

Charulata Chatterjee, PhD
Scientific head and
consultant embryologist,
Ferty9 Fertility Center.

The IVF laboratory is the epicenter of the fertility center. The dependence of patient management on laboratory data highlights the need for ensuring the quality of all procedures conducted in the lab.

We as an embryologist understand the fact and to improve outcomes we follow all standard operating procedures and quality management system. Modern accreditation programs like ISO, QAI, NABH help to achieve the best result.

Key performance indicators (KPIs) refer to a set of quantifiable measurements used to gauge a center's overall long-term performance. KPIs specifically help determine a center's strategic, financial, and operational achievements. In brief KPI means results achieved from lab procedures and goal set for improvement.

Success of fertility treatment depends on clinical work and IVF lab procedures. Hence a suitable match of clinical KPI (C-KPI) and lab KPI (L-KPI) is crucial for success. Critical health care domains like patient safety, effectiveness, equity, patient-centeredness, timeliness and efficiency is considered to establish the clinical KPI.

Key Performance Indicators (KPIs) are important for establishing minimum standards for proficiency and monitoring ongoing performance within a quality management system and aiming to achieve benchmark.

In general, the results of a series of KPIs will provide an adequate overview of the most important steps of an ART center. Three different types of indicators are reference indicator [RIs] and performance indicators [PIs] and a KPI. Suitable match of clinical KPI (C-KPI) and lab KPI (L-KPI) is crucial for success.

The six defined Performance indicators currently used for monitoring clinical work are ovarian stimulation for ART, embryo transfer, pregnancy and live birth rate. Furthermore, they can be applied to create competency profiles for clinicians.

Indicators for evaluating IVF lab result are reference indicators and performance Indicators.

Numbers of oocyte retrieved are surrogate indicators in response to ovarian stimulation and is referred as RIs. For calculating expected numbers of oocytes to be retrieved is based on total number of follicles above 14 mm size measured on ultra sound. It is useful as a measure of whether the quantity of oocytes is maximized. Again checking the percentage of mature oocytes can give an idea about stimulation regime.

Performance indicators [PIs] are referred to three arms-processes, structure and procedure outcomes. Whereas, KPIs were those related to the 'core businesses of the ART lab.

PIs of andrology lab are sperm recovery rate, and sperm motility post-wash. Post-preparation sperm motility is a valuable indicator to check the effectiveness of the sperm preparation technique.

Structure related PIs for an IVF lab includes lab staffing, lab structure, equipment and consumables which are required to efficiently deliver the service. As Cairo consensus mentions "There is only one thing that is truly important in an IVF laboratory is everything". KPI regarding IVF lab structure includes air quality in an IVF lab. Staff and equipment requirement is based on number of IVF cycles done by ART center. Consumables used for IVF lab procedure should pass all the quality measures and marked as safe to use.

Key Performance Indicators (KPIs) for an IVF lab focus on procedures such as ICSI, embryo development, pre-implantation genetic testing, and embryo freezing/warming. Common metrics include fertilization rate, oocyte degeneration rate, embryo cleavage rate, blastocyst formation rate, and embryo utilization rate.

Guidance from the Vienna consensus and Alpha consensus helps to establish these KPIs, and continuous monitoring ensures optimal lab performance. If procedures deviate from established standards, quick corrective action is essential to avoid negative outcomes.

Embryologists should calculate these KPIs monthly, and labs should set KPIs based on their individual caseload. Achieving the benchmark values derived from KPIs is desirable.

Remember, KPIs are tools for embryologists to enhance work quality. The main indicators to consider are structural, process, and procedure outcome. It's important to note that achieving a healthy live birth involves multiple disciplines and factors, all of which need to be considered in analyses.

Essential Quality Control Measures in IVF Laboratories

Dr. C. Suvarchalaa, PhD,
MSc in Clinical Embryology (Monash, Australia), MSc in Clinical Embryology(Leeds, UK), MSc in Genetics
Scientific Director ZIVA Embryology and Fertility Institute, Hyderabad

Temperature:
Considered one of the most important aspects of quality control in an IVF lab, temperature maintenance and monitoring can be very crucial during culture and handling. While culture is essentially performed at 370C, a slight variation of ±0.50C is acceptable. Oocyte spindle stability and subsequent fertilization can be compromised by a few degree fluctuations. A few critical points include:

All equipment that comes in direct or indirect contact with oocyte or
embryo i.e., laminar
work area, ICSI stage, test tube warmers, and incubators should be properly monitored.
Daily logs to be maintained of set value and actual value.
The use of properly calibrated probes/instruments is recommended.
The laminar flow temperature is to be measured in the working area by direct probe contact.
The temperature in droplets of pre-calibrated dish placed for 15 min on a working surface is
also to be checked regularly.
Pre-calibrated dish placed on Micromanipulator warm stage to be checked at immediate, 5min, and 10min. The probe should be inserted directly into the ICSI drop.
Test tubes with distilled water can be placed in test-tube warmers, and dry incubators for 2hrs for monitoring.
The temperature inside all incubators is to be checked by direct probe contact.
The temperature in the IVF lab is typically maintained between 200C to 240C.

pH:
pHi (intracellular pH) and pHe (extracellular pH) of an embryo and oocyte are essential for optimal embryo development and viability. pHi is critical for cell division, protein synthesis, cytoskeleton integrity, and membrane permeability. Oocytes have a pH of 6.89-7.12 and embryos have a pH of 7.4. pHe should be slightly higher than pHi to balance the formation of acidic ions that can occur due to intracellular metabolic processes. Therefore, to maintain the pHe/pHi gradient, commercial media have a pH range of 7.2-7.4. Minimal handling of embryos and proper functioning of incubators can ensure optimal pH conditions.
Always overlay oil over culture media and on HEPES dishes during oocyte retrieval and ICSI.
If commercially available pH meters are not available, check pH with blood gas analyzers. Measure pH at 370C as it is temperature dependent.

Osmolality:
Osmolarity and osmolality are commonly mistaken and erroneously interchanged. Osmolarity can be defined as the number of solute particles per 1 L of solvent, while osmolality is the number of solute particles in 1 kg of solvent. Osmolality has the units of Osm/kg H2O. Osmolality is measured as milliosmoles per kilogram of water (mOsm/kg H2O).
•Due to a variety in media compositions, the osmolality range of commercial embryo
culture media is 240 and 295 mOsm/kg.
•Check the water level in the incubator to maintain humidity. Low humidity can result in
evaporation leading to an increase in osmolality. High osmolality is proven to cause
irreparable damage by reducing cell division, and embryo size, and completely halting
development.
•Always cover culture dishes with oil to maintain osmolality.
•Maintain a culture drop size between 40 µl -50 µl for optimal results.
•Media making for culture should be fast and well-organized.
•Do not make media on 370C surfaces.
•Relative humidity should be between 40%-50%.

Air Quality: The Cairo consensus on the IVF laboratory and air quality has emphasized the importance of maintaining air quality for optimal results in an IVF unit. The two essential attributes are Volatile Organic Compounds (VOC) and particulate matter.

VOC: Volatile organic compounds can cause decreased embryo development and viability.
Total VOCs less than 500µg/m3 are permissible. The major sources of VOCs may be:
Ducting and AHU which could rust over a period, Laboratory uniforms, Consumables, Gases supplied to incubators, use of colognes, cleaning products, and MDF for laboratory cabinets. Dust, smoke, soot, and vehicular emissions are also detrimental. Ethanol is also a source of high VOCs.

VOCs can be reduced by:

· Reducing the exposure of embryos to the laboratory atmosphere
· Minimizing incubator door openings
· Overlaying culture media with oil
· Using paint with low VOCs in the laboratory
· Careful selection of laboratory furniture
· Off-gassing consumables
· Use of only medical-grade certified gases for incubators
· In-line filters for incubators
· Using non-porous working countertops
Daily monitoring and recording of VOCs with advanced VOC meters should be performed.

Particulate Matter

Airborne contaminants can be controlled by using HEPA filtration. A dedicated AHU unit should be installed in the central area of the lab ceiling.

• Particle matter should be less than 352,000 particles larger than 0.5 µm to 10µm per metre3.
• Positive pressure should be maintained in the laboratory.
• Installation of double doors.
• Reducing door openings for unnecessary visits to the laboratory.
• The HVAC system must run continuously.
•15-20 air changes per hour and a minimum of 3 fresh air changes are recommended.
• Inflow to be on the ceiling and outflow close to the floor.

Light:
Studies have proven that exposure of embryos to light can be detrimental to their overall growth and development. Photo-oxidation of chemical components in culture media upon exposure to light can increase reactive oxygen species that can increase toxicity levels. Light protection has a positive effect on fertilization and blastocyst formation rate.

Automation in ART Laboratories: Quality Control

Dr. Pravin Shinde
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Automation technology has become increasingly prevalent, in aspects of healthcare, including Assisted Reproductive Technology (ART) laboratories. The adoption of automation in these labs can greatly improve the quality control processes leading to reliable outcomes.

At its core automation in ART labs involves reducing the need for intervention in processes. By minimizing the potential for error and process variability automation can enhance precision and repeatability which's especially important in the delicate nature of ART work.

A significant application of automation is seen in sperm analysis. Automated sperm analyzers offer consistent measurements of parameters like concentration, motility and morphology. This reliable data aids informed decision making in the field.

Automation also benefits the vitrification of oocytes and embryos. Automated vitrification devices provide an reproducible method for cryopreservation. This standardized approach improves thaw survival rates ensuring that each oocyte or embryo has the best chance of successful preservation.

Furthermore the introduction of automated time lapse imaging systems has revolutionized embryo assessment. These systems allow for noninvasive monitoring of embryo development providing detailed morphokinetic data. This wealth of information enables embryologists to select the embryos for transfer potentially increasing the chances of a successful pregnancy.

Overall automation holds promise in improving processes and outcomes in ART laboratories. By reducing error standardizing procedures and providing data automation technology helps ensure better results, in assisted reproductive technologies.Automation has its advantages when it comes to managing data. Automated systems can. Store amounts of information, which reduces the chance of mistakes and provides a reliable record.

However there are challenges, in implementing automation in ART labs. It requires an investment and careful consideration to make sure that the automated systems meet the specific needs of each lab. In addition proper training for the staff is crucial to operate these systems.

In conclusion while automation offers opportunities to improve quality control, in ART laboratories it is not a solution that fits every situation. It is important to evaluate, plan and train in order to fully benefit from automation and ensure quality reproductive services.

The Role of Statistical Process Control (SPC) in Assisted Reproductive Technology (ART) Laboratory Quality Control

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Ensuring standards, in Assisted Reproductive Technology (ART) is incredibly important. It's crucial to achieve outcomes for patients by maintaining the reliability of laboratory procedures. One effective way to achieve this is through the use of Statistical Process Control (SPC).

SPC is a method of quality control that utilizes techniques to monitor and regulate a process. By analyzing data SPC enables laboratory staff to detect any variations that may affect the quality of the results. This proactive approach allows potential issues to be identified and addressed before they become problems.

In an ART laboratory SPC can be applied in ways. For instance it can be used to monitor the success rate of embryo development or the quality of sperm oocytes and embryos. The collected data can then be. Compared against established statistical control limits to identify any deviations from parameters.

To aid in the process control charts are commonly employed in SPC. These charts illustrate process data over time making it easier to spot trends or changes, in performance.In an ART lab setting control charts can be used to monitor parameters, like fertilization rates, blastocyst development rates and incubator temperature stability.

The main objective of implementing SPC in an ART laboratory is to improve lab processes and ultimately achieve patient outcomes. By using SPC to identify and manage sources of variation ART laboratories can ensure quality of their work. This consistency is crucial as it directly impacts the success rates of ART procedures and patient satisfaction.

To summarize SPC is a tool, for ART laboratories. It offers an data driven approach to monitor and enhance process performance ensuring the delivery of notch reproductive services.

Proficiency Testing: A Cornerstone of Quality Control in ART Laboratories

Nagasuresh Nallpati
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Radha Reddy
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INTRODUCTION

IVF embryogenesis hinges on biochemical properties of gametes and embryos, and biophysical requirements of the IVF laboratory, including design, HVAC system, equipment, and materials. Gametes and embryos, lacking epithelial surfaces and immunological defences, are susceptible to environmental factors, physiological or cellular stress that can alter gene expression or induce inheritable epigenetic effects.

Clinical parameters and the laboratory environment, materials used, procedures, and staff can all impact the process. A key consideration is lab air quality, influenced by lab design, material selection, and the gas supply system. Improving laboratory air quality through high-efficiency air filtration systems has been linked to increased birth rates.

THE INSTALLATION OF EFFECTIVE AIR QUALITY SYSTEM:

The evolution of ART labs has led to the adoption of stringent measures for air quality control, including the use of air showers, HEPA filters, and laminar air flow hoods. Key principles of air quality control in embryology labs are:

1. Air Pressure Differential: Utilizing positive pressure in the lab prevents potential pollutants from entering and causes them to be expelled outside, reducing contamination risks.

2. Turbulent Air: Positive air pressure encourages turbulent air movement, displacing stagnant air under workstations, microscopes, and other equipment. This process helps remove foreign particles that could enter the lab.

3. HEPA Filters: Highly efficient in removing particles as small as 0.3 microns (99.97% efficiency), HEPA filters can also capture smaller particles, such as the SARS-CoV-2 virus, through diffusion and interception.

4. VOC Filtration: Volatile organic compounds (VOCs) evaporate at room temperature and can combine with ozone to produce harmful byproducts. These compounds, released from various sources, can adversely impact embryo development and sperm quality. The removal of VOCs using sorption filtration, primarily through chemical filters embedded with activated carbon, is essential in embryology labs.

5. Centralized Air Filtration System: A blend of recirculated indoor and outdoor air is pressurized and filtered through dust, VOC, and HEPA filters. Maintaining a balance of 20-25% outside air and 75-80% recirculated air in an IVF lab helps optimize the use of HEPA filters, minimize operating expenses, and reduce energy waste.

KEY DETERMINANTS FOR MONITORING AIR QUALITY:

Monitoring air quality in an IVF lab is crucial to maintain a contaminant-free environment. Key considerations include airborne particle count, air pressure differential, air exchanges per hour, total VOC in air, temperature, humidity, and microbiological control.

Air Handling Unit (AHU): The AHU, a key component in an air quality system, should be properly installed, maintained, and cleaned. Negative air pressurization during AHU cleaning helps eliminate dust and other contaminants.

HEPA Filters: These expensive filters are typically replaced only if they fail inspections. Microbiological contaminants should be monitored every 3-6 months. Filter longevity, typically 2-4 years, depends on external pollution, laboratory measures to reduce particle ingress, and pressure differential monitoring.

VOC Filters: To control volatile organic compounds, use activated carbon and alumina filters impregnated with potassium permanganate. VOC levels, typically monitored using handheld probes, should be maintained below 80-100ppb, especially during key activities like incubator openings and gamete/embryo manipulation. Elevated VOC levels can significantly reduce clinical pregnancy rates.

Pre-filters: Used in the initial stage of filtration in an AHU, pre-filter replacement frequency depends on air pollutant levels and filter type. Pre-filters should be cleaned at least once every 15 days.

Temperature:

The temperature inside an IVF laboratory will be regulated by the AHU system, where the total heat output will be considered from the laboratory equipment and also staff. Range of 22-24 degree Celsius, a stable temperature should be maintained inside a laboratory. It is easier to calibrate and operate equipment when the temperature stays within a specific range.

Humidity:

40%-45% of relative humidity has to be maintained in IVF laboratory. A stable humidity is to be maintained as the lower levels of humidity can cause high levels of evaporation during dish preparation, which will impact the osmolarity of the culture medium and be detrimental to embryos in culture, while higher levels will encourage the formation of molds.

Conclusion:

In conclusion, maintaining air quality in embryology labs is crucial for successful embryogenesis. This involves regular monitoring of temperature, humidity, and pressure, and managing factors like equipment, sterile plastic ware, and multiple operators. While there's a suggested link between lab air quality and IVF success, there's no definitive method for improving air quality. Built-in systems supplying filtered air appear most effective in mitigating poor IVF outcomes due to subpar air quality, alongside good laboratory practices.

Quality Control / Quality Assurance in Pre-implantation Genetic Testing

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Preimplantation genetic testing is a procedure to evaluate genetically normal embryos and take them for embryo transfer. There are three different types of pre-implantation genetic testing. They are 1) PGT for aneuploidy (PGT-A) 2) PGT for monogenic/single gene defects (PGT-M) and 3) PGT for chromosomal structural rearrangements (PGT-SR). Quality control and Quality assurance play a major role while performing Preimplantation genetic testing (PGT) as any contamination or negligence can lead to abnormal results.

EMBRYOLOGY LABORATORY DESIGN

Embryology labs should include a dedicated biopsy area, particularly for high-workload IVF centers. This should adhere to environmental standards for air quality, positive pressure, and access. The embryo biopsy lab should be managed by an experienced embryologist knowledgeable in medical genetics. Biopsy procedures should follow standard operating procedures (SOPs) performed by skilled practitioners. Protective clothing is essential, and surfaces and equipment should be regularly cleaned with compatible disinfectants. Preparation of materials should be conducted in a dedicated clean area using UV-C light for DNA decontamination. All used solutions should be molecular grade and ready to use. Essential biopsy procedure materials include capillaries, IVF-certified dishes, mineral oil, buffered media, and micropipettes. ICSI is preferred over IVF for PGT to minimize contamination risks.

Labeling and witnessing

Witnessing is recommended during IVF-PGT procedures for accurate identification and tracking. Key stages for witnessing include:

1. Post-biopsy, to match the sample and embryo numbers.
2. During tubing, to verify the matching of sample ID and PCR tube label.
3. Placement and labeling of the embryo in the culture dish.
4. Labeling of the cryodevice, if cryopreservation is involved.
5. Post-diagnostic results, to ensure accurate correlation with the correct sample and/or embryo ID.
6. During the thawing/warming procedure and selection of normal embryos for transfer.

Handwritten identification on the cryo-support is essential when printed labels or barcodes are not possible. Even with an electronic witnessing system, a human witness is mandatory for traceability.

Setting up for a biopsy

Ensure the biopsy is performed according to established procedures by a qualified practitioner.

1. Aim to reduce the biopsy procedure duration through planning and expertise.
2. Set biopsy criteria beforehand and consistently adhere to them; regularly update as necessary.
3. Confirm micromanipulation equipment is correctly installed, calibrated, and maintained.
   Perform biopsies on a warmed stage.
4. Ensure necessary reagents and micromanipulation tools are available, sterile, and within their expiry date.
5. Prepare, equilibrate, and clearly label biopsy dishes, containing rinsing drops and a sufficient amount of

biopsy or culture medium under oil. The biopsy procedure involves creating a zona pellucida opening and removing trophectoderm cells. A hatching blastocyst is preferred as it minimizes damage and is easier to freeze. Laser is commonly used to create the opening for blastocyst biopsy due to its accuracy and speed. After biopsy, cell samples are washed, collected in small reaction tubes (tubing), and prepared for testing.

Blastocyst biopsy

Trophectoderm (TE) biopsy at the blastocyst stage allows the extraction of several cells for genetic testing without disrupting the inner cell mass (ICM) designated for fetal development. Biopsies are performed on fresh or previously cryopreserved embryos, typically on Days 5-7 post-ICSI, when the ICM is visible.

Blastocyst biopsy procedure may vary based on factors like blastocyst morphology, ICM position, and expansion rate. Typically, TE cells are aspirated gently into the biopsy pipette, with optional assistance from a laser. Laser pulses can excise aspirated cells from the blastocyst or assist in mechanical cell detachment, minimizing cell damage.

Proper tubing practices are paramount to minimize contamination and maximize amplification chances. Practitioners must wear protective clothing, including a surgical gown, hair cover, face mask, and preferably shoe covers. Gloves should be worn and replaced frequently.

Tubing requires specific training, separate from embryo biopsy training, and should be supervised by a certified clinical embryologist. Biopsied cells should be washed at least twice using a sterile transfer pipette before being moved into reaction tubes. Washing requires special care as TE cells are typically sticky, and caution is needed to avoid losing genetic material between consecutive washes. It is recommended to change denudation tips for each embryo to prevent DNA contamination.

Embryo transfer and cryopreservation.

Vitrification should be done immediately after the biopsy and before re-expansion. However, during the initial stages of the biopsy, check for survival of the blastocyst. Survival of the Blastocyst can be checked after biopsy by incubating the embryo into the culture medium and waiting for 30 minutes for a slight re-expansion of the embryo.

Re-biopsy of embryos.

If the genetic material is found to be inadequate or there's a need to reconfirm genetic analysis, re-biopsy at the blastocyst stage is suggested. The decision to re-biopsy depends on the recovery and morphology of the blastocyst. Prior to re-biopsy, the blastocyst cavity needs adequate time to re-expand. It's advisable to use the original zona pellucida (ZP) opening created during the initial biopsy, and vitrification should be carried out immediately after re-biopsy.

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In the world of embryos,
we witness the beginning
of all possibilities...

Implementing Quality Control Measures in Embryo Assessment

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Quality control in embryo assessment aims to ensure reliability and accuracy, reducing variability that could negatively impact success rates by leading to poor-quality embryo selection. By implementing quality control measures, potential bias and errors are mitigated, leading to less discrepancy in outcomes. These steps are key for preserving the integrity of research, selecting the highest quality embryos, and enhancing the effectiveness of Assisted Reproductive Technology (ART), thereby helping more couples achieve their parenting goals.

Selection of High-Quality Embryos: The primary purpose of embryo assessment is to identify and select high-quality embryos for transfer. This selection is vital because the quality of the embryo significantly influences the chances of implantation and successful pregnancy.

Optimize Success Rates: By choosing the best embryos for transfer, clinics can optimize success rates. This selection process can help reduce the number of transfer attempts needed and also reduce the risk of multiple pregnancies, which carry additional health risks for both the mother and babies.

Informed decision-making: Embryo assessment provides valuable information that helps couples and their fertility specialists make informed decisions regarding embryo transfer, cryopreservation, and the potential use of Preimplantation Genetic Testing (PGT).

Cost-effectiveness: Accurate embryo assessment can help couples avoid multiple unsuccessful ART cycles, thus saving time, emotional stress, and financial resources.

Improved success rates: By selecting embryos with the highest potential for development, embryo assessment contributes to increased success rates.

Widely followed Embryo Assessment

Gardner's Blastocyst Grading System provides embryologists and fertility specialists with a standardized approach to evaluate blastocyst quality and aids in the selection of embryos for transfer or cryopreservation.

Expansion: This refers to the degree of expansion or hatching of the blastocyst. The grading ranges from 1 (early blastocyst) to 6 (expanded blastocyst), with increasing grades indicating more advanced development.

Inner Cell Mass (ICM) quality: The ICM represents the group of cells within the blastocyst that will develop into the fetus. The ICM quality is graded as A (many tightly packed cells), B (several loosely grouped cells), or C (few cells or disorganized appearance).

Trophectoderm (TE) quality: The TE is the outer layer of cells that will form the placenta. It is graded as A (many cells forming a cohesive layer), B (several cells with slight fragmentation), or C (few cells or significant fragmentation).

1. Morphological assessment (Conventional): This is the most traditional and widely used method of embryo assessment. It involves evaluating the embryo's appearance of morphology. Key factors include:

Types of Embryo Assessment

Cell number: Embryos start as a single cell following fertilization and undergo multiple rounds of cell division. By assessing the number of cells at specific developmental stages, such as day 2 and 3 or day5, the embryologist can gather information about the embryo's growth and division rates.

Size: The size of an embryo can provide insights into its growth potential and developmental stage.

Symmetry: Symmetry refers to the uniformity and regularity of cell distribution within an embryo. Embryos with symmetrical cell division and arrangement are generally considered to have better developmental potential. Asymmetry in cell distribution or irregular cell sizes may indicate potential abnormalities or developmental issues.

Fragmentation: Fragmentation refers to the presence of small fragments or cellular debris in the embryo. It is commonly observed during embryo development, but excessive or increased fragmentation can be an indicator of poor embryo quality. Elevated levels of fragmentation are correlated with lower rates of implantation and diminished viability of embryos.

Presence of multinucleation: Multinucleation is a phenomenon characterized by the presence of multiple nuclei within the cells of an embryo. It is considered an anomaly and could potentially signify developmental or chromosomal variations in the embryo.

2. Time-lapse imaging: Time-lapse imaging is a contemporary technique that involves non-invasive monitoring of embryos, with images captured at regular intervals to avoid disrupting their environment. It allows for a more detailed evaluation of embryo development kinetics and can help identify viable embryos with a higher implantation potential. Time-lapse imaging provides additional information compared to traditional morphological assessment and reduces the need for subjective evaluation.

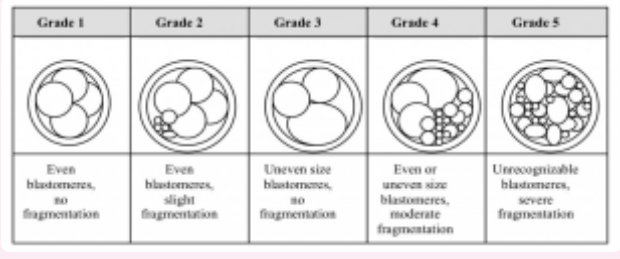
Time-lapse imaging is a contemporary technique that involves non-invasive monitoring of embryos, with images captured at regular intervals to avoid disrupting their environment.

3. Preimplantation Genetic Testing (PGT): PGT is a functional assessment method that involves analyzing the genetic material of embryos to detect chromosomal abnormalities or specific genetic disorders.

4. Metabolomic profiling: This is an emerging technology where the substances used or produced by the embryo in the culture medium are analyzed. This can provide information about the embryo's metabolic health and potential for successful implantation

5. Secretome analysis: Secretome analysis focuses on evaluating the substances secreted by embryos, such as growth factors and cytokines, into the culture media. These secreted molecules can reflect the metabolic and physiological status of the embryo.

6. Proteomic and transcriptomic analysis: These methods involve evaluating the expression of specific proteins or genes within embryos. They are capable of providing information regarding the developmental competence of the embryo and its potential for successful implantation. Nevertheless, these techniques are currently in the research phase and have not yet gained widespread usage in clinical practice. By adhering to standardized protocols and guidelines, the risk of misdiagnosis is reduced, ensuring the accurate selection of viable embryos for transfer or cryopreservation.



Impact of key factors like temparature on IVF culture media on embryo growth and subsequent events

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KPIs (Key Performance Indicators) are set up to evaluate the overall performance of any IVF laboratory, these may include Sperm motility, Fertilization rate, blastocyst formation rate, implantation rate, etc... Before setting up the KPIs it is a prerequisite to ensure that the laboratory culture conditions are optimum and healthy with proper maintenance and quality checks time to time. The goal of any IVF establishment is to provide the gametes or embryos with conditions that mimic the female reproductive tract and produce embryos that are as capable of as the embryos grown in vivo.

The key factor that are thrown into light in this short review are:

Temperature

Maintaining temperature at optimum levels is of utmost importance.

Many intracellular processes like Metabolism, protein synthesis, mitochondrial functions, and maintaining cytoskeletal structure, etc., are sensitive to Temperature.

Temperature:

Temperature control is critical in handling human gametes and embryos due to their sensitivity to thermal shocks. Not only can cellular functions, such as metabolism, transportation, and various phases of the cell cycle be temperature-dependent, but culture media supplements like proteins, amino acids, and buffers can also be damaged or denatured.

Cells naturally have mechanisms that respond to potential causes of stress. In the case of thermal stress, cells produce specialized proteins known as heat shock proteins (HSPs) that manage cell functions. The activity of HSPs depends on the level and duration of stress, and damage within their threshold level. If stress is short-lived, cellular mechanisms effectively manage by activating genes responsible for heat-labile mRNA stabilization, along with the production of HSPs. However, if stress is prolonged and beyond the cell's threshold capacity, genes responsible for inhibiting cell cycle and facilitating apoptosis are upregulated, while downregulating HUR protein.

Studies on mouse embryos have demonstrated that embryos subjected to temperature variations showed a slower growth rate, poor blastocyst morphology, and increased expression of apoptotic genes, compared to the control group. Meanwhile, oocyte spindles are extremely sensitive to temperature changes. Therefore, maintaining an ideal temperature while handling gametes and embryos is of paramount importance.

A study conducted by Xiao-Fang Sun1 2004 et al observed that the oocytes spindles started to disassemble at 39oC for 20mins but a faster disassembly and displacement of the spindle in the Ooplasm when they are exposed to 40oC. This indicates that the spindle assembly is more impacted by higher temperatures than that of the lower side.

Although the spindle arrangement was recovered in most of the cases morphologically it was not the same.

Membrane permeability is also influenced by temperature it is directly proportional i.e. the permeability increases with temperature. In vitrification, it depends on the route of exchange between water and cryoprotectants. Whatever it might be we should avoid exposing them to higher temperatures while vitrification as the cryoprotectants would become toxic to embryos and oocytes at higher temperatures.(Keisuke Edashige 2017)

Temperature share an inverse relationship; as temperature rises, pH decreases, making the environment more acidic. Conversely, as temperature falls, the environment becomes more basic. This relationship directly impacts the conditions of an IVF culture.

Although it's generally accepted that the average human body temperature is around 37°C, this isn't an absolute normal. The ideal temperature can vary based on individual factors, such as sex, and environmental conditions like region, altitude, and surrounding habitat.

The optimal temperature for an IVF culture system is a topic of debate, but it's widely accepted that a range of 37°C +/- 1 is ideal. This standard is maintained in almost all IVF incubators worldwide. However, as incubators are man-made machines, they may malfunction. It's the responsibility of embryologists to ensure backup systems are in place and that regular quality control checks and preventive maintenance are performed to ensure optimal conditions.

Preparations —The Unseen Vim of QC –

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The IVF industry has been effectively enhancing the standard of care and the live birth rate since the 1980s. On the other hand, many clinics find it difficult to improve their mediocre success rates to excellent ones. Even if we are inclined to become experts in IVF procedures like ICSI or embryo transfer, we must first familiarize ourselves with the process that serves as the basis for all of those procedures. IVF procedures must be carried out precisely, but preparations—the invisible strength that is sometimes neglected—are equally crucial.

Taking steps to ensure you have sufficient storage and streamlining QA/QC are two examples of preparation!

Preparations for ICSI or IVF, though seemingly mundane, are vital and usually handled by trainees or junior embryologists. These include managing media, making dishes based on expected follicles, aliquoting, and maintaining disposable supplies. Extra steps like those in Embryo Transfer (ET) or freezing might also be necessary. However, one must be careful not to mistakenly use non-CO2-based media for dishes meant to culture gametes in a CO2 incubator, as this could lead to complications.

Apart from media preparation, the following activities are also important in the IVF or ICSI preparation process:

a) Monitor consumables and media for expiration dates.
b) Even with an AMC contract, check expiration dates on equipment filters.
c) Ensure all equipment functions properly to prevent issues.
d) Maintain the cold chain consistently.
e) Regularly check stock and refill cryo tanks with liquid nitrogen.

Gametes and embryos are sensitive to "reprotoxicity," an adverse impact on their physiology and viability potentially caused by toxic substances in disposable plastic items used in IVF procedures. Even a 2% decrease in embryo viability from each of the 30 pieces of polystyrene used can result in a 44% lower implantation viability. To mitigate this, use disposables and media that have undergone competence testing, ensuring their safety for use with human gametes and embryos.

IVF certification certifies instruments as safe after rigorous testing, often on mouse embryos. Still, using untested vessels for aliquoting media and oil can introduce reprotoxic substances. Therefore, only lab equipment proven safe for IVF procedures should be used, and it's essential to be cautious about the vessels used for aliquoting, avoiding those untested or not designed for IVF operations.

By keeping quality control (QC) and quality assurance (QA) in mind, there are safe ways to prepare dishes and aliquot media.

Controlling temperature, osmolality, and pH, three essential components of cell homeostasis is of the utmost importance for quality assurance in the IVF lab. In contrast to the standardization of temperature and osmolality control, pH management continues to be essentially discretionary. The correlation between the bicarbonate concentrations in the culture media and the laboratory incubator's CO2 setting is crucial for identifying the optimum pH setting for culture media. This is reliant on atmospheric pressure and, until recently, on the humidity of the air used to nurture embryos (new advancements in CO2 calibration technology have reduced the relevance of humidity).

Temperature regulation is key in working with media for IVF or ICSI procedures, as evaporation can affect osmolality. This process accelerates with frequent bottle openings and storage at room temperature, causing the media to dry up and possibly failing to meet required standards. Hence, it's advised to minimize the time the bottle is kept at room temperature and keep it closed as much as possible.

The components most affected by evaporation are ammonium and amino acids. If bottles are left outside the refrigerator, ammonium levels will rise, amino acid degradation will speed up, and the medium quality will worsen. Therefore, adopting an aseptic method when using the same bottle for multiple days is critical to prevent contamination and minimize evaporation.

There are two of the many straightforward methods to lessen evaporation:

Start preparing dishes immediately after taking the media out of the refrigerator.

Never use a bottle that has warmed to room temperature.

Labeling of Labware: Dishes have a specific label or barcode area that is separate from the handling area to guarantee patient identity.

A tapered edge: Tapered edges facilitate access because they are also readily visible at the edge of the well. Dishes should only be made of IVF-approved polymers.

When considering all of the before mentioned considerations, including osmolality, non-toxic vessels for aliquoting, and aseptic work practices, it is possible to safely aliquot media and utilize the complete shelf life as long as you are aware of the risks and take steps to reduce the contamination.

Get your preparations right, the results will definitely follow! !

Why QA/QC is essential in an IVF Lab?

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We being embryologists are happy, at our place, positive till our IVF lab KPI is above competency levels and we have good rates of ongoing pregnancies. The trouble in paradise arises when our Usable Blastocyst Rate is low and this in turn will affect the pregnancy and implantation rate. This can happen from the oldest IVF lab to a new IVF lab.

What are the possible factors we could check for troubleshooting? The first and foremost, factor is "the Incubator". If and when any IVF lab is functioning, daily checks of incubator Gas levels, water levels, and temperature are essential. Cleaning the incubator and getting it calibrated by a qualified engineer every 3-4 months is mandatory to allow the incubator to function at its optimum.

The next factors to be checked are Lab Humidity, Temperature, and VOC levels. The oocyte and the sperm are meant to be in the Human body and the body temperature is 37 degrees Celsius.

Lab instruments perform at their optimum when the room temperature is between 23 - 250 C. Hence, lab temperature must be maintained between 23 - 25 degrees Celsius. What happens if the temperature goes below this - the heating plates of the ICSI manipulator and the heating stage of LAF will not be able to maintain 37 degree Celsius which in turn will affect the gametes which will affect the embryos and in turn the lab KPI rates.

Next comes Relative Humidity (RH) – the ideal RH of the lab is around 45-50%. If it goes above then there will be fogging seen on instruments and heating plates; also it would create difficult working conditions which in turn would hamper the proficiency of the embryologists. VOCs are tricky to manage in any IVF lab. More people entering the IVF lab, Hygiene of people entering the IVF lab, and use of perfume or any other cosmetics leads to an increase in VOC. This can be reduced by regular intervals of lab cleaning and having an AHU system in place. If VOC levels are high in the lab it could lead to embryos having a high percentage of fragmentation.

The next important factor is Culture Medica. Its QC can be done by performing a sperm survival test for each new batch. Maintaining records of a batch of media used and embryo utilization rates. If a sudden drop is seen with a new batch of culture media it is advisable to stop using it and check by using a different batch media.

The most important factor is undoubtedly the embryologist who needs to do a regular QA/AC check. Performance levels can be analyzed by comparing the rates among embryologists. Embryologists being dedicated and in the right mind is extremely important for seeing a good embryo utilization rate.

Let's all strive to work towards making IVF journey a success for each and every couple who walks into our clinic.

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How Hygienic is the Air in your IVF lab?

Hemanth Valluri
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An IVF lab's air quality will inevitably have an impact on embryonic growth, which has an important effect on IVF success. Numerous sources may produce airborne toxic substances and a significant amount of pollutants, such as heavy metals, nitrous oxide, sulfur dioxide, and carbon monoxide, can be found in the air in urban areas.

Indoor sources of volatile organic compounds (VOCs) include construction materials such as medium-density fiberboard (MDF), PVC flooring, paints, adhesives,
cleaning solutions, floor waxes, cosmetics, and cigarette smoke. These can contribute to "sick building syndrome." Despite the lack of evidence about their effect on embryos in in vitro fertilization (IVF), health and safety authorities recommend safe VOC exposure limits for adults and provide building ventilation guidelines.

Location has a key role in minimizing air pollution.

Designing a lab requires careful consideration of its location for optimal air quality. Air intakes should be away from pollution sources like busy roads or parking lots. The lab should be situated within a building where it's not exposed to contaminants from other departments like laundry or sterilization. If possible, the building should be in an area with low pollution. These factors need to be thought through in the early design phase to ensure air quality in the lab. Better results are guaranteed in a clean-room setting. If the culture environment has such a significant impact on embryo development and implantation, the IVF laboratory would do well to adhere to stricter regulatory norms for particle control and, in particular, molecular air quality.

A tightly regulated, sealed atmosphere is created using "clean room technology," in which the introduction of particles and pollutants is greatly reduced. This is accomplished by flushing highly filtered air under positive pressure through High Efficiency Particulate Air (HEPA) filters, which results in a cleaner atmosphere overall.

Filters that use chemical or absorbent filtering, UV light irradiation, photocatalytic conversion, or a combination of some or all of the aforementioned techniques are new to the IVF sector and are contained within the ducts. Clean room approval should be given to all furniture, cleaning procedures, and attire.

In laboratory design, walls should be smooth, non-porous, and easy to clean, possibly using aluminum trihydrate panels. The lab should have sufficient sinks and drainage, with sealed lighting to reduce airborne particles. Ducts and pipes should be concealed to minimize dust, which can be done using modular walls. Standalone, prefabricated Clean Room Cells are a cost-effective way to create a cleanroom environment, especially in areas with poor air quality. Lastly, medical-grade gases should be transported using inert stainless steel tubing due to copper's tendency to oxidize.

Don't overlook the rooms adjacent to the lab.

It's crucial to consider adjacent rooms when designing a lab. For procedures requiring collaboration with an operating room, like oocyte retrieval and embryo transfer, hermetically sealed doors and pass-through windows can help maintain clean air. The nearby changing rooms should be kept impeccably clean. The adjacent operating room should maintain the same positive pressure as the lab. Post-construction, a removable sealed part of the wall can facilitate the entry of large lab equipment like incubators and laminar flow cabinets, especially during construction and off-gassing stages. Once operational, ensure doors are large enough for the movement of such equipment.

Maintain restricted access

Lab access should be restricted to reduce contamination, barring unapproved individuals and objects. An air lock chamber can help minimize particle contamination, serving as a physical barrier and an "air shower." Staff hygiene is critical, as they can be a significant source of pollution. Special clean environment clothing can protect against particle and microbiological contamination. These polyester outfits act as particle shields and don't shed fibers. When laundering, ensure minimal residual laundry detergent to prevent contamination.

Microbial count can be calculated using a swab test.

Tactical swab testing of all workstations and important locations to evaluate microbial count is another metric that can detect contamination from the operator (i.e., from perspiration or breaches in hygiene practices) and should be added to your entire quality control system. Microbial load on lab surfaces is counted using agar contact plates or kits that are readily available. Utilizing KPIs like the in vitro fertilization rate, cleavage rate, embryo quality, and pregnancy rate will improve your overall quality management.

Maintaining proper laboratory practices to ensure good air quality

Maintaining good lab practices is crucial for preserving clean air. This includes regular monitoring of air flow parameters like filter integrity and daily positive pressure measurements, and equipment maintenance to prevent air quality decline due to depleted HEPA filters. Daily recording of pressure differential can show filter effectiveness and monitor positive pressure between the lab and adjacent rooms. Regular use of particle counters can assess air quality, and VOC levels can be measured affordably with organic chemical sensors like ACS badges or electronic devices to monitor exposure to harmful vapors.

QMS for Preservation of Sperm Functions in routine lab procedures

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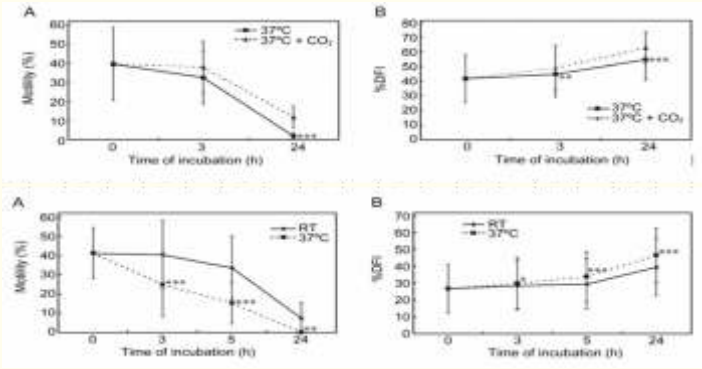
Sperm Function is a collective term for various activities that a sperm can orchestrate in order to be a part of successful ongoing pregnancy. Each of its activity has importance in the process of natural conception. Although we overcome some of these issues with ART but they are the potential indicators of sperm quality. Sperm functions are vulnerable to external stimuli. In ART, sperm is subjected to stressful processes like centrifugation and immobilization which may jeopardize its functions to some extent or total loss. This article explains 3 such scenarios where sperm functions get altered in due process and related preventive measures for better treatment outcomes.

1. Room Temperature can protect the Sperm Motility and can slowdown the rate of DNA fragmentation

Rising patient numbers at Assisted Reproductive Technology (ART) clinics have led to an increased number of unattended samples. The improper handling and storage of these samples can negatively impact sperm quality. Delays in processing liquefied semen or culture media have become unavoidable, sometimes leading to a total loss of sperm motility.
Long-term in-vitro incubation can damage sperm quality and motility. Post-ejaculation, semen samples should ideally be stored in a 37C incubator for 15-20 minutes for liquefaction. Prolonged incubation can cause the semen to dehydrate and become hyperviscous, reducing sperm quality and motility, leading to poor fertilization, implantation failure, or miscarriage. Impaired liquefaction is a factor in infertility, affecting an estimated 12% of couples trying to conceive naturally.



RT- Room Temperature; * Significant Change**

Motility & DFI analysis of Sperm in Raw Sample [4]: Prolonged Incubation of sperm in Liquefied Semen at 37C shows a rapid decrease in motility(A) from the time of liquefaction. Similarly, a significant increase in DFI(B) observed. A similar drop of motility(A) and increase in DFI(B) observed with incubation at 37C with or without CO2.

Conclusions: Preserving semen samples at room temperature (20-37C) post-liquefaction can safeguard motility and minimize sperm DNA damage. Studies have shown significant quality deterioration post 3 hours when incubated at 37C. The complex constitution of semen samples can auto-degrade, a process accelerated by temperature. The semen matrix includes proteins, sugars, lipids, metabolites, enzymes, and cytokines, among other things. Any alteration in its makeup can directly impact sperm and its functions, which is why it's standard practice to separate sperm from this matrix promptly after liquefaction. While temperature aids liquefaction, it's not necessary post 30-40 minutes or post-liquefaction. Hence, samples should be removed from incubators and kept at room temperature until processed.

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2. Subpopulation of Low DFI fraction of sperms can be obtained with DGC + Swim-Up

Sperm DFI is known high with increased abstinence period, liquefaction time, concentration and decreased motility and normal forms [b,4]. Out of these variables a random semen sample can present tens of thousands of permutations along with its composition, physical and rheological parameters. Since we knew sperm DFI is a crucial factor that determines the successful implantation or miscarriages all our SOPs are constructed to prevent DNA fragmentation such as providing stable pH with HEPES buffered media, separating swim-up fraction post 30mins of incubation, and providing constant temperature of 37C till its usage. But there are certain things that can result high levels of DFI in the final preparation than the original neat sample.

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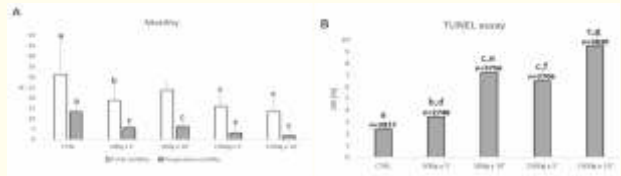

Besides the temperature and Ph, centrifugation has a direct effect on motility (A) and DFI (B) of the resultant yield. Shorter the duration of centrifugation & centrifugal force better the post process motility and DNA integrity [5].

Conclusions: The necessity for sperm separation in-vitro is mainly due to detrimental effect of its semen composition when left in contact. Since most of the ART procedures require few good sperms for therapeutic use neither whole sample nor high rpm is required. Soon after liquefaction sperm can be prepared and kept for incubation with suitable media until use. The ultimate aim should be segregation of sperms with intact DNA which can be achieved with less swim-up time. If the samples are unattended for more than an hour without separating the swim-up fraction might lose its purpose. Repeating centrifugation steps might not help since they promote DNA fragmentation due to ROS, and disrupting membrane integrity.

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bWHO laboratory manual for the examination and processing of human semen Sixth Edition
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4.Asian J Androl. 2010 Sep; 12(5): 753–759. Published online 2010 Jun 21 doi: 10.1038/aja.2010.46 PMCID: PMC3739315 PMID: 20562894 Preparation and incubation conditions affect the DNA integrity of ejaculated human spermatozoa Rieko Matsuura, Takumi Takeuchi, and Atsumi Yoshida
5.Centrifugation Force and Time Alter CASA Parameters and Oxidative Status of Cryopreserved Stallion Sperm Giuseppina Marzano 1,2,3, Natalina Moscatelli 4,5, Mariangela Di Giacomo 4 , Nicola Antonio Martino 6,7, Giovanni Michele Lacalandra 8 , Maria Elena Dell'Aquila 6 , Giuseppe Maruccio 1,2 , Elis Improvement of motility after culture of testicular spermatozoa: the effects of incubation timing and temperature Akram Hosseini, Mohammad Ali Khalili

Embryology - The Beast to Beauty.

Dr. G. Divyashree,
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As a woman embryologist, my heart aches to say no to cosmetics in this per diem life, as everyone wants to be groomed and good-looking, especially women, who are considered beauty conscious by the world. Yet here I am, happy to say I chose such a field of science where I feel like lending a hand to God's most intricate creation on earth.

Cosmetics include a tonne of chemicals and have a serious detrimental effect on women's reproductive health, according to IVF experts. They also have negative impacts on IVF labs by changing the Volatile Organic Compounds (VOCs) there, which are necessary for the orderly development of eye-catching embryos. Quality control (QC) is a paramount feature of IVF lab operations and is essential in maintaining the uncompromised quality of embryos.

We are aware that embryos are extremely sensitive to environmental factors, and VOCs are not an exception. Volatile organic compounds (VOCs) are organic substances that are hydrocarbon bases that have a high vapor pressure at room temperature and are detrimental to the development of an embryo. The VOCs directly bind to DNA, abort embryonic development, and are linked to more DNA fragmentation in human sperm. The embryologist or any lab workers in the IVF suite should avoid these VOCs producing items in use because they are already transmitted through a variety of items such as lab tools, furnishings, fragrances, smoking, etc. and we shouldn't be adding to the burden. .

Additionally, VOCs are believed to be lethal to the embryos. For the least amount of physical and chemical stress on gametes, blastocyst development, and clinical pregnancy rates, the best acceptable upper limit for VOCs is 0.5 ppm. To make sure the lab is operating optimally, it is important to regularly validate the air quality and calculate key performance indicators (KPIs) such as the rates of in vitro fertilization, cleavage, blastocyst formation, embryo quality, and pregnancy.

We as embryologists need to be more cautious so that we don't adopt unhealthy work habits and contribute to the increase in VOCs. We should be cautious and deliberate in our daily lifestyle decisions.

Most of the cosmetic products are prohibited from use in IVF labs:

- Cosmetics, nail polishes, and perfumes
-Fragrant cosmetics (foundations, lipsticks, and anti-aging creams)
- Conditioners and shampoos with aroma Biological Soaps

Various chemicals in cosmetics, such as acetaldehyde, toluene, parabens, and formaldehyde, can disrupt embryogenesis by altering VOC levels, causing oxidative and physiological stress. These can impact how embryonic genes are expressed or regulated and potentially cause imprinting and epigenetic effects that may pass down generations.

However, not all VOCs, such as silicones used in incubator gaskets and tubing, or high molecular weight alkanes like paraffinic oils, are harmful to gametes or embryos.

In ART laboratories, typical VOCs include ethanol and propanol. Other VOC-producing chemicals include acetone and formaldehyde, which, even at low levels (0.03ppm), can impede human embryos' development to the blastocyst stage. Similarly, acetaldehyde and hexaldehyde can negatively affect human embryos, leading to arrest at four-cell or pre-blastocyst stages.

Cell division, essential for embryogenesis, can be disrupted by oxidative stress caused by Reactive Oxygen Species (ROS). These ROS can harm proteins and macromolecules through lipid peroxidation, DNA oxidation, and apoptosis. Various capabilities, such as the substance's ability to enter the culture system, impact gametes, embryos, and blastocysts, or the biological ability to metabolize, detoxify, and excrete contaminants, can be inhibited.

Differences in solubility and reactivity can reverse the movement of molecules between water and oil or air and water. Technology plays a vital role here, helping reduce pollution and exposure in the lab by employing HVAC-AHU and gas supply filters.

Maintaining an efficient IVF laboratory involves regular monitoring of VOC levels, temperature, and humidity. Any variations can result in high VOC levels detrimental to embryonic development. To ensure optimal conditions, lab protocol should prohibit perfumes, lotions, cosmetics, or lipsticks, which can disrupt embryonic growth. For a sterile environment, an HVAC-AHU system should be in place to ensure good air quality. Moreover, lab staff should wear scent-free, autoclaved clothing, hair caps, and facemasks while in the lab.

We, embryologists, don't need to be beautiful from the exterior for our success as professionals; instead, we need skilful hands and generous hearts.

The smaller details we overlook do matter!!!

Jayasree.A,
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When we hear the phrase "Quality control" the most common assumption or the image that comes to our mind is probably something related to incubators and culture systems. Even though culture systems and instruments are a very significant and noteworthy part of quality control, it's important to know that there are additional crucial components beyond that to QC like laboratory environment which includes air quality, temperature, humidity, air pressure, calibration and maintenance of instruments, handling of gametes and embryos, SOPs, etc. Qc is a set of protocols that makes sure that the fundamentals in an IVF unit are working according to their specified threshold.

LABORATORY ENVIRONMENT:

One of the widely practiced concepts in foreign countries to ensure quality control is the "clean room technology. A clean room is a well-contained and restricted environment in which the airborne particles are free from environmental air contaminants, microbes, and VOCs, and the air pressure, humidity, temperature, and lighting is controlled. Now this might sound just like a traditional IVF laboratory that 90% of centres use. But the key difference between a traditional IVF laboratory and a clean room laboratory is the specific protocols that are implemented to maintain a low level of contamination.

The Setup clean room environment:

The setup of the clean room environment includes the division of the cleanroom into

3 different rooms, the dry room, the ovum pickup room, and the embryo transfer room, and finally the semen processing room. An efficient IVF facility comprises a cleanroom, an ovum pickup room, an embryo transfer room, and a semen preparation room. The cleanroom, which is devoid of any equipment, acts as a buffer between the IVF OT and the IVF lab and is equipped with air filters, temperature control, and pressure control.

The ovum pickup room and the embryo transfer room should be adjacent to the cleanroom, connected via a heated pass-through hatch. This hatch allows the safe transfer of follicular fluid in tubes and the sterile packaging of the ET catheter. The person in the cleanroom should be well-trained and familiar with the procedures, as they handle the transfer of gametes and embryos between the IVF lab and IVF OT.

Lastly, the semen preparation room is dedicated to wet diagnostics and sperm sample preparation. All tests involving cell fixation and staining should occur outside the cleanroom to prevent biocide spread. After patient sample collection, only the processed semen sample in a sterile tube should enter the cleanroom; the collection container, potentially contaminated, should never be near the cleanroom or OPU room.

The entry protocol for personnel in an IVF centre is meticulous. Initially, staff must remove their clothing before entering the pre-changing area, where they replace their shoes, thoroughly wash hands, and wear necessary items such as a hood, facemask, and beard mask.

Next, they enter the changing area to don clean room disposable clothing, including shoe covers, hair caps, and masks, without touching the floor. Any non-essential items, including food, beverages, mobile phones, handbags, and more, are strictly prohibited in the IVF clean room.

Once inside, staff walk on a sticky clean room mat and change shoes again, followed by disinfecting hands with an IVF-approved product. Cost shouldn't be the main factor when choosing hand hygiene products. Staff with skin or respiratory conditions should be advised not to work in the clean room until their health improves.

Traditional laboratories that adapted the clean room has witnessed optimized outcomes. Better embryo quality, higher live birth rates, and lower miscarriage rates are being noted after the switch to a clean-room IVF facility. Working in an IVF clean room differs substantially from working in a traditional IVF laboratory. It can be a little overwhelming and exhausting with the number of steps involved in the decontamination protocols to maintain this low level of contamination. It is practically impossible to change the setup of the IVF, but we can always implement a few smaller changes according to the clean room.

Because In The End, Small Changes Do Make A Big Difference.

S.No.	Clinic Level	Name of the Organisation	Email ID
1	Level-2	Dr.radhikas fertility and surgical center	drradhikasfertility@gmail.com
2	Level-2	Sree nandaka fertility and laparoscopy hospital	sreenandakafertility@gmail.com
3	Level-2	Rekhasagar ivf and research center	drrekharaniakula@gmail.com
4	Level-2	Art clinic	jyothitesttubebabycenter@gmail.com
5	Level-2	Wish fertility	wishfertilitynzb@gmail.com
6	Level-2	Birthright Fertility by Rainbow Hospital - Kondapur	brfrh.kp@rainbowhospitals.in
7	Level-2	Birthright Fertility by Rainbow Hospital - Kondapur	karuna.p@rainbowhospitals.in
8	Level-2	Revive clinics and fertility Center	revivefertility@gmail.com
9	Level-2	Aadya fertility center	drsujithareddy31@gmail.com
10	Level-2	Indira ivf hospital private limited	centerhead.hyderabad@indiraivf.in
11	Level-2	Dr.Padmaja Institute for Fertility Center	vinaybura5@gmail.com
12	Level-2	Sree Raghavendra Fertility centre	sreeraghavendrafertility@gmail.com
13	Level-2	Ferty9 fertility center(a unit of star fertility pvt.ltd.)	jhansi@ferty9.com
14	Level-2	Kadimi ivf center	kadimihospitalnlg@gmail.com
15	Level-2	Janani Fertility centre	jananifertilitycentrehyd@gmail.com
16	Level-2	IVF Access, Hyderabad a unit of PN IVF Access Private Limited	jbh.art@ivfaccess.com
17	Level-2	Fertivision health care pvt.ltd.	chary@ferty9.com
18	Level-2	Maa fertility centre and hopsital	themaafertility@gmail.com
19	Level-2	Southern gem hospital	drsweta.agarwal@yahoo.com
20	Level-2	Morpheus kasturi international IVF center	kalpanaathuru@gmail.com
21	Level-2	Ferty9 fertility center (a unit of star fertility pvt.ltd.)	meenakshi@ferty9.com
22	Level-2	Shouryas testtube baby center	saishouryahospital@gmail.com
23	Level-2	Nova ivf fertility banjara hills	nova.banjarahills@novaivffertility.com
24	Level-2	Nova ivf fertility kukatpally	nova.kukatpally@novaivffertility.com
25	Level-2	Ziva embryology and fertility institute	info@zivafertility.com
26	Level-2	Ziva embryology and fertility institute	KOMPALLY@zivafertility.com
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29	Level-2	Sree fertility & gyane centre private ltd	
30	Level-2	Sree fertility & gynae centre private ltd	
31	Level-2	Aeva fertility	aevafertility@gmail.com

32	Level-2	Sumuka Fertility center	mumsfertility@gmail.com
33	Level-2	Iswarya fertility centre	hyd@iswarya.in
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36	Level-2	Lush fertility	lushfertility@gmail.com
37	Level-2	Vijaya fertility centre	vijayabharati102@gmail.com
38	Level-2	Ira fertility and women health care	irafertility22@gmail.com
39	Level-2	Wings zoya ivf	WINGSZOYAIVF@GMAIL.COM
40	Level-2	Hyderabad women and fertility centre	hyderabadfertility1@gmail.com
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48	Level-2	Ova fertility centre	ovafertilitycentre@gmail.com
49	Level-2	Prashanthi ivf clinic	prashanthiivfcentre7191@gmail.com
50	Level-2	Siri fertility center	srilathahospital@gmail.com
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52	Level-2	Sreelatha fertility ivf center	sreelathaivf@gmail.com
53	Level-2	Genesis test tube baby centre	drksswamy503001@gmail.com
54	Level-2	Dr.lalitha fertility & laparoscopic center	dr.lalithafertiliyart@gmail.com
55	Level-2	Sreelaxmi test tube baby multi speciaity hospital	sreelaxmihospital@gmail.com
56	Level-2	Eva IVF (A Unit of Prashanthi Centre for Fertility PVT LTD)	accounts@evaivf.in
57	Level-2	Dr.Archana Fertility and Laparoscopy Center	archuomc@gmail.com
58	Level-2	Kamala Fertility (a unit of srinivasa hospiral);(ART Clinic)	kamalafertility@gmail.com
59	Level-2	Surya fertility ivf icsi & iui center	drkothagattu@gmail.com
60	Level-2	Sree havisha hospital	sreehavishareception@gmail.com
61	Level-2	Kamala Fertility (a unit of Srinivasa Hospital),ART clinic	srinivasahospitalhyt@gmail.com
62	Level-2	G.b.r hospital & fertility	gbrhospital4s.2005@gmail.com
63	Level-2	Geetanjali test tube baby center (art clinic)	geetanjali_ivf@yahoo.com
64	Level-2	Sri venkateshwara nursing home	vamshi2611@gmail.com
65	Level-2	Amrutha nursing home	amruthaivf@gmail.com
66	Level-2	Shreshta fertility centre	shreshtafertilitycenter@gmail.com
67	Level-2	Avi fertility & laproscopic centre	avifertility@gmail.com
68	Level-2	Prasad Hospitals India pvt ltd	sumaindia@gmail.com

69	Level-2	Akshaya fertility clinic	drmeera1962@gmail.com
70	Level-2	Abhijay fertility hospital private limited	art_hyderabad@arcfertility.in
71	Level-2	Sudha fertility and day care centre	accounts.hyd@sudhahospitals.com
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77	Level-2	Secunderabad womens clinic and infertility center	info@swcic.com
78	Level-2	Anusri hospitals	anusrihospitals@gmail.com
79	Level-2	Iswarya fertility services private limited	ivfhyderabad11@gmail.com
80	Level-2	Swapna health care	accounts.swapnahealthcare@gmail.com
81	Level-2	Motherhood fertility centre	motherhoodfertility@gmail.com
82	Level-2	Aaroos Fertility Center	aaroosfertility@gmail.com
83	Level-2	Mother hospitals and ivf centre	kuttiailapuram7@gmail.com
84	Level-2	Dharmaraju test tube baby centre	chittagiri@yahoo.co.in
85	Level-2	Aanvi Fertility and Womens Centre	aanviivf@gmail.com
86	Level-2	V r test tube baby center	skreddy24@yahoo.in
87	Level-2	Srijan fertility centre	kumarkoka@yahoo.com
88	Level-2	Plan b fertility	suchitahealthcare2022@gmail.com
89	Level-2	Plan B Fertility (A Unit of Suchita Healthcare and Research Pvt Ltd)	drdhatrikumari@gmail.com
90	Level-2	Konark hospital	konarkfertilitycentre@gmail.com
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92	Level-2	M/s. Sadguru healthcare services pvt ltd (oasis fertility)	art.tlck@oasisindia.in
93	Level-2	Tulasi ivf center	tulasiivfcenter@gmail.com
94	Level-2	Shreya fertilty centre	shreyafertilitycentre@gmail.com
95	Level-2	Rohan fertility centre	rohannursinghome@gmail.com
96	Level-2	Department of ART-Unit of Sundari Maternity and General Hospital	deepthiivf@gmail.com
97	Level-2	Life- lakshmi's advanced fertility centre	lifebylakshmi@gmail.com
98	Level-2	Tina fertility and IVF centre	drnitheshatina@gmail.com
99	Level-2	Mathrushree fertility centre	neeraj.isac@gmail.com
100	Level-2	Sree havisha hospital	sreehavishahospital@gmail.com
101	Level-2	Ferty9 fertility center (a unit of star fertility pvt. Ltd.)	naveen@ferty9.com
102	Level-2	Sri CS memorial hospital extension	aishaarav22@icloud.com

103	Level-2	Sri CS Memorial Hospital Extension block	aishaarav22@gmail.com
104	Level-2	Iswarya health private limited	warangal@iswarya.in
105	Level-2	Iswarya health private limited	anand@iswarya.in
106	Level-2	Angels fertility	asmaayesha1975@gmail.com
107	Level-2	Anu fertility and contraception services and research institute pvt ltd	anutesttubebaby@gmail.com
108	Level-2	Art Fertility Clinics, a unit of Global Fertility Solutions Private Limited	arthyd.legal@artfertilityclinics.com
109	Level-2	Avira fertility center	avirafertilitycenter@gmail.com
110	Level-2	Avni fertility andrology	avnifertility@gmail.com
111	Level-2	Babys life Hospital	ivfblhpraveena@gmail.com
112	Level-2	Bluegene pvt ltd	prasadinfertilitysolutions@gmail.com
113	Level-2	Dr Neerajas fertility and gynaec center	neervalli@gmail.com
114	Level-2	Dr padmaja fertility centre & nursing home	info@drpadmajaivf.com
115	Level-2	Dr Praveena fertility centre	jsuresh972@gmail.com
116	Level-2	Dr vasavis hospital center for fertility and birth	drvasavishospital@gmail.com
117	Level-2	Dr. Bhavani fertility centre	drbhavanifertilitycentre@gmail.com
118	Level-2	Dr.lalitha fertility&laparoscopic center	dr.lalithafertility123@gmail.com
119	Level-2	M/s Sadguru Healthcare Services Pvt Ltd	art.bh1@oasisindia.in
120	Level-2	M/s ,Sadguru Health Care Services Pvt Ltd	art.dsnr@oasisindia.in
121	Level-2	Oasis Fertility (A Unit of Sadguru Healthcare Services Pvt Ltd)	art.kngr@oasisindia.in
122	Level-2	M/s Oasis Center for Reproductive Medicine (A Unit of Sadguru Healthcare Services Pvt Ltd)	art.sb@oasisindia.in
123	Level-2	Mamata fertility hospital(a unit of infertility institute & research centre pvt ltd	art@mamatafertility.com
124	Level-2	Esha ivf fertility center	chandanalakkireddi@gmail.com
125	Level-2	Genesis Fertility & Laparoscopy Centre	doctors@genesisfertilitycentre.co.in
126	Level-2	Mom ivf and research center pvt ltd	dr.poornimakanth@gmail.com
127	Level-2	Medical Health and Research Institute	drroyarozati@gmail.com
128	Level-2	Hegde Hospital	drvandana@hegdehospital.com
129	Level-2	Hira fertility centre	fazz.encantador143@gmail.com

130	Level-2	Ganasreesai ivf center	gssivf@gmail.com
131	Level-2	Institute of Women Health and Fertility	healthandfertility@gmail.com
132	Level-2	Hyderabad women and fertility center	hyderabadfertility01@gmail.com
133	Level-2	Mira fertility	info.mirafertility@gmail.com
134	Level-2	Eva IVF	info@evaivf.in
135	Level-2	Sai Kiran Hospital (A Unit of Kiran Infertility Centre Pvt Ltd)	info@kiranivfgenetic.com
136	Level-2	KIMS fertility centre	kimsfertilitycentre3@gmail.com
137	Level-2	Kamineni fertility centre	life@kamineni fertility.com
138	Level-2	Felicity Ivf and Fertility Center	maddireddysumanth@gmail.com
139	Level-2	Ziva embryology and fertility institute	manikonda@zivafertility.com
140	Level-2	Sai Matrika Fertility Centre	matrikaivf@yahoo.com
141	Level-2	Medicover healthcare pvt ltd	medicover.hydlegal@medicoverfertility.com
142	Level-2	Rohit test tube baby centre	muvvachandu@yahoo.com
143	Level-2	Juhi fertility centre	nopa56@gmail.com
144	Level-2	Esha ivf fertility center	prabhat.lakki@gmail.com
145	Level-2	Ferty9 fertility center (a unit of star fertility pvt.ltd.)	rahmatunnisa@ferty9.com
146	Level-2	Virinchi health care private limited	ravinder.a@virinchi.com
147	Level-2	Ziva embryology and fertility institute	sanathnagar@zivafertility.com
148	Level-2	Om birth fertility centre	sandhyareddydodda@gmail.com
149	Level-2	Nest fertility and ivf	shravani.pulluri89@gmail.com
150	Level-2	Ferticare ivf asist	shrutimanvikar@gmail.com
151	Level-2	M/s. 9m fertility centre (a unit of ankura fertility centre llp)	sivalingamaneni@ankurahospital.com
152	Level-2	Sreefertility & gyane centre private ltd	sreefertilitykdp@gmail.com
153	Level-2	Origin fertility clinic & research center	syed@originfertilitycenter.com
154	Level-2	Jaiswal multi speciality hospital	umajaiswal25@gmail.com
155	Level-2	Womb fertility and maternity centre	wombfertility.maternitycentre@gmail.com

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